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(54) Antigenically active proteins and peptides, and transmissible gastro-enteritis virus TGEV vaccines.

(57) The invention relates to new antigenic proteins which can be used to stimulate the immunity of pigs against transmissible gastro-enteritis virus (TGEV)-vaccines, and to fragments or derivatives thereof. The invention also relates to vaccines which comprise such antigenic proteins or peptides.

The nucleotide sequence and amine acid sequence of a gene coding for protein of an TGEV-strain are indicated in figure 1.

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New antigenically active proteins and peptides, and transmissible gastro-enteritis virus (TGEV)-vaccines.

The invention relates to a new antigenic protein which is capable of stimulating the immunity of pigs against transmissible gastro-enteritis virus (TGEV), and to fragments or derivatives thereof. The invention further relates to vaccines which comprises such an antigenic protein or peptide.

Transmissible gastro-enteritis is an infection disease of the intestine in pigs causing high mortality in piglings younger than 2 weeks and hence great economic losses to the pig breeders. Inspite of the existance of various types of vaccines, the problem of TGE has not been solved effectively. Therefore, the development of new, better vaccines against TGE is urgently necessary.

The cause of TGE is a virus which belongs to the group or corona viruses. Corona viruses are positive single-stranded RNA viruses having a lipid envelope. The TGEV genome RNA has a length of approximately 23,600 bases. The virus particle (virion) comprises three important proteins, namely the peplomeric protein (S), the nucleocapside protein (N), and the matrix protein (M).

The characteristic protrusions at the surface of the virion are formed by the peplomeric protein (S). Each peplomer is formed by a glycopolypeptide. The protein is fixed in the membrane by means of a terminal membrane anchor sequence. It is known from literature that antibodies generated against the peplomeric protein of the said virus, provide protection to the pig.

According to the present invention the nucleotide sequence and the amino acid sequence of the TGEV Purdue strain were determined.

The invention therefore relates to proteins and fragments thereof which comprise at least one of the areas which play a part in the protection against TGE infections.

Theoretically, such fragments can be used as such as an antigen for vaccinating against TGE. However, it is to be preferred to couple said fragments to a suitable carrier.

The peptide fragments or proteins according to the invention which comprise at least one antigenically active peptide fragment can be prepared according to methods known for the preparation of peptides and proteins.

First of all, the peptides can be prepared synthetically by means of known techniques starting from the individual amino acids or smaller peptide fragments.

The peptides can also be obtained biosynthetically using recombinant DNA techniques and expression systems, for example, by:

a) transformation of host cells with an expression vector which comprises a DNA, coding for an (the) antigenic determinant(s) (peptides in general);

b) expressing the genome inserted in the expression vector;

c) harvesting the cell culture, and

d) separating the synthesised peptide (protein).

The invention therefore also relates to a method of preparing a DNA molecule which codes for peptides according to the invention. Such a method comprises the folllowing steps:

a) isloation of TGE single-stranded RNA;

b) synthesis of a cDNA strand complementary to the RNA strand mentioned in a); and

c) removal of the RNA molecule and synthesis of a second cDNA strand, using the first cDNA strand as a template.

The double-stranded DNA obtained in this manner may be incorporated in an expression vector in known manner so that a recombinant expression vector is formed. This vector may be introduced into a suitable host cell, for example, by transformation.

The invention will now be described in greater detail with reference to the ensuing specific example.

EXAMPLE

a) Virus and tissue culture.

TGEV strain Purdue was purified twice by means of pricking of a plaque on PD5 cells. Culture conditions were described previously in L. Jacobs et al, J. Virol. 57; (1986); 1010-15.

b) Isolation of subgenomic mRNA's

PD5 cells (9×10^7) were infected with an infection multiplicity of 15. Seven hours after infection actinomycine D was added and 10 hours after infection the cells well lysed and the RNA extracted, as described previously by W.J.M. Spaan et al., Virol. 108 (1981); 424-434. The RNA (560 µg) was dissolved in a buffer having a high salt concentration (10 mM Tris-HCl pH 7.5, 0.05% SDS, 500 mM NaCl) and transferred to an oligo(dT)-cellulose column for a selection on poly(A)+ RNA. After elution with 10 mM Tris-HCl pH 7.5the RNA was concentrated by precipitation with ethanol and

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fractionated by means of an isokinetic sucrose gradient (B.A.M. van der Zeijst et al., in G.D. Fasman (Ed.), Handbook of biochemistry and molecular biology, 3rd ed. Physical and Chemical data. Vol. 1 CRC Press Inc., Boca Raton, Fla., (1976), pp. 426-519). Before providing the RNA on the gradient it was dissolved in 50 mM Tris-HCl pH 7.4, 10 mM LiCl, 1% SDS and heated at 56°C for 1 minute. ^{32}P-labelled TGEV mRNA's were added as markers. The identification and translation of TGEV mRNA's is described by Jacobs et al., 1986.

c) Cloning of TGEV peplomer (E2) gene.

RNA3 obtained after purification over the sucrose gradient was used for the synthesis of cDNA (carried out as described by Gubler and Hoffman (Gene, 25 (1983), 263-269). Calf-thymus pentamers were used as primers and methyl mercury hydroxide for denaturing the RNA. The double-stranded cDNA was elongated with dC residues and then cloned in a pUC9 vector elongated with dG residues. The DNA was used for the transformation of E. coli strain JM109 as described by D. Hanahan et al., J. Mol. Biol., 166, (1983), 557-580. In this manner a cDNA library of approximately 900 transformants was obtained. The plasmides comprise insertions having a length up to 5kb.

d) Selection of E2-specific recombinants.

E2-specific recombinants could be identified by using the homology between TGEV and FIPV. Two restriction fragments from the E2 gene of FIPV were labelled with ^{32}P-dATP by means of nick translation and were used for the selection of the recombinants. Twenty-four recombinants hybridised with probe 2, eight with probe 1 and two recombinants hybridised with both probes. A restriction map of three recombinants was made. The recombinants which represent the whole peplomer gene were used for sequence analysis. Restriction fragments were separated in an agarose gel and isolated by means of an NA45 membrane (Schleicher and Schuell) and cloned in M13 vectors (mp8 and/or mp19). Sequence analysis was carried out according to the dideoxy-nucleotide termination method of Sanger et al., Proc. Nath. Acad. Sci. USA 74, (1977), 721-732. Both the universal M13 primer and E2-specific primers were used. The data were analysed by means of a DEC20/60 computer and the programs of Staden, Nucl. Acid. Res. 10, (1982), 4731-4751.

e) Nucleotide sequence.

The nucleotide sequence of the peplomer gene of TGEV (Purdue strain) is shown in figure 1. The sequence comprises an open reading frame of 4347 nucelotides with a coding capacity for a precursor protein of 1449 amino acids (nucleotide position 155-4502). Upstream of the ATG codon and downstream of first stop codon a recurring sequence (ACTAAACT) is present. This "intergenic" sequence also flanks the E2 gene of FIPV and has also been found upstream of the nucleocapsid gene of TGEV and MHV.

f) Amino acid sequence.

The ATG translation initiation codon (position 161) is succeeded by 15 amino acids which probably form the cleavable signal peptide, two hydrophilic amino acids succeeded by 13 hydrophobic amino acids. The extreme carboxy terminal part, position 4319-4393 (25 amino acids) comprises a hydrophobic region which presumable serves as a transmembrane anchor, both regions are underlined in Figure 1.

Claims

1. A gene which codes for protein of transmissible gastro-enteritis virus (TGEV), characterized by the nucelotide sequence as shown in Figure 1.

2. A protein, characterized by the amino acid sequence as shown in Figure 1, or a part thereof.

3. An immunogen, characterized in that it comprises a protein or peptide as claimed in Claim 2, whether or nor coupled to a carrier.

4. A vaccine, characterized in that it comprises an immunogen as claimed in Claim 3.

5. A method of preparing a protein or peptide as claimed in Claim 2, characterized in that

a) the protein or peptide is synthesised in a manner known per su from individual amino acids and/or by coupling smaller peptides; or

b) a host cell is transformed with an expression vector which comprises a DNA coding for a protein or peptide as claimed in Claim 2, and the genome introduced in the expression vector is expressed.

6. A DNA molecule which comprises a nucleotide sequence which codes for a protein or peptide as claimed in Claim 2, or which codes for a polypeptide which comprises a protein or peptide as claimed in Claim 2.

7. A recombinant DNA expression vector which expresses the whole protein or peptide or a part thereof as defined in Claim 2, comprising an

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operon with initiator sequences and terminator sequence and a nucleotide sequence which codes for the said protein or peptide, the said nucleotide sequence being situated between the initiator sequence and the terminator sequence of the operon.

8. A host cell which comprises a recombinant DNA expression vector and/or DNA molecule as claimed in Claim 6 or 7.

9. A method of preparing a DNA molecule which codes for a protein or peptide as claimed in Claim 2, characterized in that

a) single-stranded TGEV-RNA is isolated;

b) a cDNA strand complementary to said RNA strand is synthesised;

c) the RNA strand is removed and a second strand of cDNA is synthesised with the first cDNA as a template.

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 *  H  T  M  K  K  L  F  V  V  L  V  V  M  P  L  I  Y  G  D
TTAACACACCATGAAAAAACTATTTGTGGTTTTGGTCGTAATGCCATTGATTTATGGAGA
       160        170        180        190        200        210

 N  F  P  C  S  K  L  T  N  R  T  I  G  N  Q  W  N  L  I  E
CAATTTTCCTTGTTCTAAATTGACTAATAGAACTATAGGCAACCAGTGGAATCTCATTGA
       220        230        240        250        260        270

 T  F  L  L  N  Y  S  S  R  L  P  P  N  S  D  V  V  L  G  D
AACCTTCCTTCTAAACTATAGTAGTAGGTTACCACCTAATTCAGATGTGGTGTTAGGTGA
       280        290        300        310        320        330

 Y  F  P  T  V  Q  P  W  F  N  C  I  R  N  N  S  N  D  L  Y
TTATTTTCCTACTGTACAACCTTGGTTTAATTGCATTCGCAATAATAGTAATGACCTTTA
       340        350        360        370        380        390

 V  T  L  E  N  L  K  A  V  Y  W  D  Y  A  T  E  N  I  T  W
TGTTACACTGGAAAATCTTAAAGCAGTGTATTGGGATTATGCTACAGAAAATATCACTTG
       400        410        420        430        440        450

 N  H  R  Q  R  L  N  V  V  V  N  G  Y  P  Y  S  I  T  V  T
GAATCACAGACAACGGTTAAACGTAGTCGTTAATGGATACCCATACTCCATCACAGTTAC
       460        470        480        490        500        510

 T  T  R  N  F  N  S  A  E  G  A  I  I  C  I  C  K  G  S  P
AACAACCCGCAATTTTAATTCTGCTGAAGGTGCTATTATATGCATTTGTAAGGGCTCACC
       520        530        540        550        560        570

 P  T  T  T  T  E  S  S  L  T  C  N  W  G  S  E  C  R  L  N
ACCTACTACCACCACAGAATCTAGTTTGACTTGCAATTGGGGTAGTGAGTGCAGGTTAAA
       580        590        600        610        620        630

 H  K  F  P  I  C  P  S  N  S  E  A  N  C  G  N  M  L  Y  G
CCATAAGTTCCCTATATGTCCTTCTAATTCAGAGGCAAATTGTGGTAATATGCTGTATGG
       640        650        660        670        680        690

 L  Q  W  F  A  D  E  V  V  A  Y  L  H  G  A  S  Y  R  I  S
CCTACAATGGTTTGCAGATGAGGTTGTTGCTTATTTACATGGTGCTAGTTACCGTATTAG
       700        710        720        730        740        750

 F  E  N  Q  W  S  G  T  V  T  F  G  D  M  R  A  T  T  L  E
TTTTGAAAATCAATGGTCTGGCACTGTCACATTTGGTGATATGCGTGCGACAACATTAGA
       760        770        780        790        800        810
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 V  A  G  T  L  V  D  L  W  W  F  N  P  V  Y  D  V  S  Y  Y
AGTCGCTGGCACGCTTGTAGACCTTTGGTGGTTTAATCCTGTTTATGATGTCAGTTATTA
      820        830        840        850        860        870

 R  V  N  N  K  N  G  T  T  V  V  S  N  C  T  D  Q  C  A  S
TAGGGTTAATAATAAAAATGGTACTACCGTAGTTTCCAATTGCACTGATCAATGTGCTAG
      880        890        900        910        920        930

 Y  V  A  N  V  F  T  T  Q  P  G  G  F  I  P  S  D  F  S  F
TTATGTGGCTAATGTTTTTACTACACAGCCAGGAGGTTTTATACCATCAGATTTTAGTTT
      940        950        960        970        980        990

 N  N  W  F  L  L  T  N  S  S  T  L  V  S  G  K  L  V  T  K
TAATAATTGGTTCCTTCTAACTAATAGCTCCACGTTGGTTAGTGGTAAATTAGTTACCAA
     1000       1010       1020       1030       1040       1050

 Q  P  L  L  V  N  C  L  W  P  V  P  S  F  E  E  A  A  S  T
ACAGCCGTTATTAGTTAATTGCTTATGGCCAGTCCCTAGCTTTGAAGAAGCAGCTTCTAC
     1060       1070       1080       1090       1100       1110

 F  C  F  E  G  A  G  F  D  Q  C  N  G  A  V  L  N  N  T  V
ATTTTGTTTTGAGGGTGCTGGCTTTGATCAATGTAATGGTGCTGTTTTAAATAATACTGT
     1120       1130       1140       1150       1160       1170

 D  V  I  R  F  N  L  N  F  T  T  N  V  Q  S  G  K  G  A  T
AGACGTCATTAGGTTCAACCTTAATTTTACTACAAATGTACAATCAGGTAAGGGTGCCAC
     1180       1190       1200       1210       1220       1230

 V  F  S  L  N  T  T  G  G  V  T  L  E  I  S  C  Y  T  V  S
AGTGTTTTCATTGAACACAACGGGTGGTGTCACTCTTGAAATTTCATGTTATACAGTGAG
     1240       1250       1260       1270       1280       1290

 D  S  S  F  F  S  T  G  E  I  P  F  G  V  T  D  G  P  R  Y
TGACTCGAGCTTTTTCAGTTACGGTGAAATTCCGTTCGGCGTAACTGATGGACCACGGTA
     1300       1310       1320       1330       1340       1350

 C  Y  V  H  Y  N  G  T  A  L  K  Y  L  G  T  L  P  P  S  V
CTGTTACGTACACTATAATGGCACAGCTCTTAAGTATTTAGGAACATTACCACCTAGTGT
     1360       1370       1380       1390       1400       1410
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 K  E  I  A  I  S  K  W  G  H  F  Y  I  N  G  Y  N  F  F  S
CAAGGAGATTGCTATTAGTAAGTGGGGCCATTTTTATATTAATGGTTACAATTTCTTTAG
      1420       1430       1440       1450       1460       1470

 T  F  P  I  D  C  I  S  F  N  L  T  T  G  D  S  D  V  F  W
CACATTTCCTATTGATTGTATATCTTTTAATTTGACCACTGGTGATAGTGACGTTTTCTG
      1480       1490       1500       1510       1520       1530

 T  I  A  Y  T  S  Y  T  E  A  L  V  Q  V  E  N  T  A  I  T
GACAATAGCTTACACATCGTACACTGAAGCATTAGTACAAGTTGAAAACACAGCTATTAC
      1540       1550       1560       1570       1580       1590

 K  V  T  Y  C  N  S  H  V  N  N  I  K  C  S  Q  I  T  A  N
AAAGGTGACGTATTGTAATAGTCACGTTAATAACATTAAATGCTCTCAAATTACTGCTAA
      1600       1610       1620       1630       1640       1650

 L  N  N  G  F  Y  P  V  S  S  S  E  V  G  L  V  N  X  S  V
TTTGAATAATGGATTTTATCCTGTTTCTTCAAGTGAAGTTGGTCTTGTCAATAAGAGTGT
      1660       1670       1680       1690       1700       1710

 V  L  L  P  S  F  Y  T  H  T  I  V  N  I  T  I  G  L  G  M
TGTGTTACTACCTAGCTTTTACACACATACCATTGTTAACATAACTATTGGTCTTGGTAT
      1720       1730       1740       1750       1760       1770

 K  R  S  G  Y  G  Q  P  I  A  S  T  L  S  N  I  T  L  P  M
GAAGCGTAGTGGTTATGGTCAACCCATAGCCTCAACATTAAGTAACATCACACTACCAAT
      1780       1790       1800       1810       1820       1830

 Q  D  H  N  T  D  V  Y  C  I  R  S  D  Q  F  S  V  Y  V  H
GCAGGATCACAACACCGATGTGTACTGTATTCGTTCTGACCAATTTTCAGTTTATGTTCA
      1840       1850       1860       1870       1880       1890

 S  T  C  K  S  A  L  W  D  N  I  F  K  R  N  C  T  D  V  L
TTCTACTTGCAAAAGTGCTTTATGGGACAATATTTTTAAGCGAAACTGCACGGACGTTTT
      1900       1910       1920       1930       1940       1950

 D  A  T  A  V  I  K  T  G  T  C  P  F  S  F  D  K  L  N  N
AGATGCCACAGCTGTTATAAAAACTGGTACTTGTCCTTTCTCATTTGATAAATTGAACAA
      1960       1970       1980       1990       2000       2010

 Y  L  T  F  N  K  F  C  L  S  L  S  P  V  G  A  N  C  K  F
TTACTTAACTTTTAACAAGTTCTGTTTGTCGTTGAGTCCTGTTGGTGCTAATTGTAAGTT
      2020       2030       2040       2050       2060       2070
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 D  V  A  A  R  T  R  T  N  E  Q  V  V  R  S  L  Y  V  I  Y
TGATGTAGCTGCCCGTACAAGAACCAATGAGCAGGTTGTTAGAAGTTTGTATGTAATATA
      2080       2090       2100       2110       2120       2130

 E  E  G  D  N  I  V  G  V  P  S  D  N  S  G  V  H  D  L  S
TGAAGAAGGAGACAACATAGTGGGTGTACCGTCTGATAATAGTGGTGTGCACGATTTGTC
      2140       2150       2160       2170       2180       2190

 V  L  H  L  D  S  C  T  D  Y  N  I  Y  G  R  T  G  V  G  I
AGTGCTACACCTAGATTCCTGCACAGATTACAATATATATGGTAGAACTGGTGTTGGTAT
      2200       2210       2220       2230       2240       2250

 I  R  Q  T  N  R  T  L  L  S  G  L  Y  Y  T  S  L  S  G  D
TATTAGACAAACTAACAGGACGCTACTTAGTGGCTTATATTACACATCACTATCAGGTGA
      2260       2270       2280       2290       2300       2310

 L  L  G  F  K  N  V  S  D  G  V  I  Y  S  V  T  P  C  D  V
TTTGTTAGGTTTTAAAAATGTTAGTGATGGTGTCATCTACTCTGTAACGCCATGTGATGT
      2320       2330       2340       2350       2360       2370

 S  A  Q  A  A  V  I  D  G  T  I  V  G  A  I  T  S  I  N  S
AAGCGCACAAGCAGCTGTTATTGATGGTACCATAGTTGGGGCTATCACTTCCATTAACAG
      2380       2390       2400       2410       2420       2430

 E  L  L  G  L  T  H  W  T  T  T  P  N  F  Y  Y  Y  S  I  Y
TGAACTGTTAGGTCTAACACATTGGACAACAACACCTAATTTTTATTACTACTCTATATA
      2440       2450       2460       2470       2480       2490

 N  Y  T  N  D  R  T  R  G  T  A  I  D  S  N  D  F  D  C  E
TAATTACACAAATGATAGGACTCGTGGCACTGCAATTGACAGTAATGATTTTGATTGTGA
      2500       2510       2520       2530       2540       2550

 P  V  I  T  Y  S  N  I  G  V  C  K  N  G  A  F  V  F  I  N
ACCTGTCATAACCTATTCTAACATAGGTGTTTGTAAAAATGGTGCTTTTGTTTTTATTAA
      2560       2570       2580       2590       2600       2610

 V  T  H  S  D  G  D  V  Q  P  I  S  T  G  N  V  T  I  P  T
CGTCACACATTCTGATGGAGACGTGCAACCAATTAGCACTGGTAATGTCACGATACCTAC
      2620       2630       2640       2650       2660       2670

 N  F  T  I  S  V  Q  V  E  Y  I  Q  V  Y  T  T  P  V  S  I
AAACTTTACCATATCCGTGCAAGTCGAATATATTCAGGTTTACACTACACCAGTGTCAAT
      2680       2690       2700       2710       2720       2730
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 D  C  S  R  Y  V  C  N  G  N  P  R  C  N  K  L  L  T  Q  Y
AGACTGTTCAAGATATGTTTGTAATGGTAACCCTAGGTGTAACAAATTGTTAACACAATA
      2740       2750       2760       2770       2780       2790

 V  S  A  C  Q  T  I  E  Q  A  L  A  M  G  A  R  L  E  N  M
CGTTTCTGCATGTCAAACTATTGAGCAAGCACTTGCAATGGGTGCCAGACTTGAAAACAT
      2800       2810       2820       2830       2840       2850

 E  V  D  S  M  L  F  V  S  E  N  A  L  K  L  A  S  V  E  A
GGAGGTTGATTCCATGTTGTTTGTTTCTGAAAATGCCCTTAAATTAGCATCTGTTGAAGC
      2860       2870       2880       2890       2900       2910

 F  N  S  S  E  T  L  D  P  I  Y  K  E  W  P  N  I  G  G  S
ATTCAATAGTTCAGAAACTTTAGACCCTATTTACAAAGAATGGCCTAATATAGGTGGTTC
      2920       2930       2940       2950       2960       2970

 W  L  E  G  L  K  Y  I  L  P  S  H  N  S  K  R  K  Y  R  S
TTGGCTAGAAGGTCTAAAATACATACTTCCGTCCCATAATAGCAAACGTAAGTATCGTTC
      2980       2990       3000       3010       3020       3030

 A  I  E  D  L  L  F  D  K  V  V  T  S  G  L  G  T  V  D  E
AGCTATAGAGGACTTGCTTTTTGATAAGGTTGTAACATCTGGTTTAGGTACAGTTGATGA
      3040       3050       3060       3070       3080       3090

 D  Y  K  R  C  T  G  G  Y  D  I  A  D  L  V  C  A  Q  Y  Y
AGATTATAAACGTTGTACAGGTGGTTATGACATAGCTGACTTAGTATGTGCTCAATACTA
      3100       3110       3120       3130       3140       3150

 N  G  I  M  V  L  P  G  V  A  N  A  D  K  M  T  M  Y  T  A
TAATGGCATCATGGTGCTACCTGGTGTGGCTAATGCTGACAAAATGACTATGTACACAGC
      3160       3170       3180       3190       3200       3210

 S  L  A  G  G  I  T  L  G  A  L  G  G  G  A  V  A  I  P  F
ATCCCTTGCAGGTGGTATAACATTAGGTGCACTTGGTGGAGGCGCCGTGGCTATACCTTT
      3220       3230       3240       3250       3260       3270

 A  V  A  V  Q  A  R  L  N  Y  V  A  L  Q  T  D  V  L  N  K
TGCAGTAGCAGTTCAGGCTAGACTTAATTATGTTGCTCTACAAACTGATGTATTGAACAA
      3280       3290       3300       3310       3320       3330

 N  Q  Q  I  L  A  S  A  F  N  Q  A  I  G  N  I  T  Q  S  F
AAACCAGCAGATTCTGGCTAGTGCTTTCAATCAAGCTATTGGTAACATTACACAGTCATT
      3340       3350       3360       3370       3380       3390
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 G  K  V  N  D  A  I  H  Q  R  S  R  G  L  A  T  V  A  K  A
TGGTAAGGTTAATGATGCTATACATCAAAGATCACGAGGTCTTGCTACTGTTGCTAAAGC
      3400       3410       3420       3430       3440       3450

 L  A  K  V  Q  D  V  V  N  I  Q  G  Q  A  L  S  H  L  T  V
ATTGGCAAAAGTGCAAGATGTTGTCAACATACAAGGGCAAGCTTTAAGCCACCTAACAGT
      3460       3470       3480       3490       3500       3510

 Q  L  Q  N  N  F  Q  A  I  S  S  S  I  S  D  I  Y  N  R  L
ACAATTGCAAAATAATTTCCAAGCCATTAGTAGTTCTATTAGTGACATTTACAATAGGCT
      3520       3530       3540       3550       3560       3570

 D  E  L  S  A  D  A  Q  V  D  R  L  I  T  G  R  L  T  A  L
TGACGAATTGAGTGCTGATGCACAAGTTGACAGGCTGATCACAGGAAGACTTACAGCACT
      3580       3590       3600       3610       3620       3630

 N  A  F  V  S  Q  T  L  T  R  Q  A  E  V  R  A  S  R  Q  L
TAATGCATTTGTGTCTCAGACTCTAACCAGACAAGCGGAGGTTAGGGCTAGTAGACAACT
      3640       3650       3660       3670       3680       3690

 A  K  D  K  V  N  E  C  V  R  S  Q  S  Q  R  F  G  F  C  G
TGCCAAAGACAAGGTTAATGAATGCGTTAGGTCTCAGTCTCAGAGATTCGGATTCTGTGG
      3700       3710       3720       3730       3740       3750

 N  G  T  H  L  F  S  L  A  N  A  A  P  N  G  M  I  F  F  H
TAATGGTACACATTTGTTTTCACTCGCAAATGCAGCACCAAATGGCATGATTTTCTTTCA
      3760       3770       3780       3790       3800       3810

 T  V  L  L  P  T  A  Y  E  T  V  T  A  W  P  G  I  C  A  S
CACAGTGCTATTACCAACGGCTTATGAAACTGTGACTGCTTGGCCAGGTATTTGTGCTTC
      3820       3830       3840       3850       3860       3870

 D  G  D  R  T  F  G  L  V  V  K  D  V  Q  L  T  L  F  R  N
AGATGGTGATCGCACTTTTGGACTTGTCGTTAAAGATGTCCAGTTGACTTTGTTTCGTAA
      3880       3890       3900       3910       3920       3930

 L  D  D  K  F  Y  L  T  P  R  T  M  Y  Q  P  R  V  A  T  S
TCTAGATGACAAGTTCTATTTGACCCCCAGAACTATGTATCAGCCTAGAGTTGCAACTAG
      3940       3950       3960       3970       3980       3990

 S  D  F  V  Q  I  E  G  C  D  V  L  F  V  N  A  T  V  S  D
TTCTGACTTTGTTCAAATTGAAGGGTGCGATGTGCTGTTTGTTAATGCAACTGTAAGTGA
      4000       4010       4020       4030       4040       4050
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 L  P  S  I  I  P  D  Y  I  D  I  N  Q  T  V  Q  D  I  L  E
TTTGCCTAGTATTATACCTGATTATATTGATATTAATCAGACTGTTCAAGACATATTAGA
      4060       4070       4080       4090       4100       4110

 N  F  R  P  N  W  T  V  P  E  L  T  F  D  I  F  N  A  T  Y
AAATTTTAGACCAAATTGGACTGTACCTGAGTTGACATTTGACATTTTTAACGCAACCTA
      4120       4130       4140       4150       4160       4170

 L  N  L  T  G  E  I  D  D  L  E  F  R  S  E  K  L  H  N  T
TTTAAACCTGACTGGTGAAATTGATGACTTAGAATTTAGGTCAGAAAAGCTACATAACAC
      4180       4190       4200       4210       4220       4230

 T  V  E  L  A  I  L  I  D  N  I  N  N  T  L  V  N  L  E  W
CACTGTAGAACTTGCCATTCTCATTGACAACATTAACAATACATTAGTCAATCTTGAATG
      4240       4250       4260       4270       4280       4290

 L  N  R  I  E  T  Y  V  K  W  P  W  Y  V  W  L  L  I  G  L
GCTCAATAGAATTGAAACCTATGTAAAATGGCCTTGGTATGTGTGGCTACTAATAGGCTT
      4300       4310       4320       4330       4340       4350

 V  V  I  F  C  I  P  L  L  L  F  C  C  C  S  T  G  C  C  G
AGTAGTAATATTTTGCATACCATTACTGCTATTTTGCTGTTGTAGTACAGGTTGCTGTGG
      4360       4370       4380       4390       4400       4410

 C  I  G  C  L  G  S  C  C  H  S  I  C  S  R  R  Q  F  E  N
ATGCATAGGTTGTTTAGGAAGTTGTTGTCACTCTATATGTAGTAGAAGACAATTTGAAAA
      4420       4430       4440       4450       4460       4470

 Y  E  P  I  E  K  V  H  V  H  *  I  *  N  V  N  S  I  I  C
TTACGAACCAATTGAAAAAGTGCACGTCCATTAAATTTAAAATGTTAATTCTATCATCTG
      4480       4490       4500       4510       4520       4530

 Y  N  S  S  C  F  C  *  R  I  L  L  R  M  M  N  K  V  F  K
CTATAATAGCAGTTGTTTCTGCTAGAGAATTTTGTTAAGGATGATGAATAAAGTCTTTAA
      4540       4550       4560       4570       4580       4590

 N  *  T
GAACTAAACT
      4600
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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 20 0045

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, vol. 185, no. 3, 1985, pages 63-82; S. HU et al.: "Studies of TGEV spike protein GP195 expressed in E. coli and by a TGE-vaccinia virus recombinant" * Whole article *	1-9	C 12 N 15/00 A 61 K 39/225 C 12 P 21/02 C 12 N 1/20
X,D	JOURNAL OF VIROLOGY, vol. 57, no. 3, March 1986, pages 1010-1015, American Society for Microbiology; L. JACOBS et al.: "Characterization and translation of transmissible gastroenteritis virus mRNAs" * Page 1014 *	2,3	
A	IDEM	1,5-9	
A	US-A-4 335 105 (P.M. GOUGH) * Whole document *	2-4	
X,P	THE JOURNAL OF GENERAL VIROLOGY, vol. 68, no. 7, July 1987, pages 1883-1890, SGM; D. RASSCHAERT et al.: "The predicted primary structure of the peplomer protein E2 of the porcine coronavirus transmissible gastroenteritis virus" * Whole article, especially figure 2 *	1-9	TECHNICAL FIELDS SEARCHED (Int. Cl.4) C 12 N A 61 K C 12 P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14-04-1988	CUPIDO M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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